(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 December 2003 (04.12.2003)

PCT

(10) International Publication Number
WO 03/099806 A1

(51) International Patent Classification[7]: C07D 339/04, C07C 279/14, A61K 7/48, 31/385, A61P 39/06

(21) International Application Number: PCT/IT03/00316

(22) International Filing Date: 23 May 2003 (23.05.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
RM2002A000296 27 May 2002 (27.05.2002) IT

(71) Applicant *(for all designated States except US)*: **LICREA S.R.L.** [IT/IT]; Via Parmenide, s.c., I-04013 Latina LT (IT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BUONONATO, Antonietta** [IT/IT]; Via della Balduina, 128, I-00136 Roma RM (IT). **FESTUCCIA, Andrea** [IT/IT]; Via di Val Tellina, 116, I-00151 Rome RM (IT).

(74) Agent: **RAIMONDI, Adriana et al.**; Cavattoni-Raimondi, Viale dei Parioli, 160, I-00197 Roma RM (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/099806 A1

(54) Title: CREATINE SALT HAVING ENHANCED NUTRITIONAL, ANTIOXIDANT AND THERAPEUTIC EFFICACY AND COMPOSITIONS CONTAINING SAME

(57) Abstract: A novel creatine salt and the compositions containing same as active principle, such as dietary supplements, dietetic products, nutraceuticals, cosmetic and drugs, are disclosed.

Creatine salt having enhanced nutritional, antioxidant and therapeutic efficacy and compositions containing same.

The present invention relates to a novel, stable and non-hygroscopic creatine salt having enhanced nutritional, antioxidant and therapeutic efficacy and to a process for preparing same and also relates to the compositions which can be used as energizing dietary supplements, nutraceuticals, health foods, cosmetics and drugs containing said salt as active ingredient.

More particularly, such novel compound is the salt of creatine with lipoic acid which hereinbelow will be referred to as creatine lipoate.

EP 0 702 953 B1 (Asta Medica A.G.) discloses dosage forms (presentations) for use as pharmaceuticals or food additives, such as tablets, capsules, suppositories and the like, containing physiologically acceptable, solid salts of α-lipoic acid. These presentations are endowed with faster release and enhanced bioavailability of the active principle than those comprising non-salified α-lipoic acid only as active principle.

The list of bases suitable for salifying α-lipoic acid to give the aforesaid solid salts comprises several thousands of potential reactants since it encompasses, *inter alia*, "amines of the formula NR1R2R3 in which the radicals R1, R2 and R3 are identical or different and denote hydrogen, C1-C4 alkyl or C1-C4 oxyalkyl, alkylenediamines with an alkylene chain of 2 to 6 C atoms, saturated cyclic aminocompounds with 4-6 ring-forming carbon atoms."

Among the various other bases, in addition to essential and non essential basic aminoacids, also the non-protein-forming aminoacid creatine is mentioned.

It should be noted, however, that the preparation of any specific α-lipoic acid salt is not shown and that the physico-chemical properties of any such salt are totally missing. Only the sodium and trometamol salts are specifically mentioned as components of various presentations.

Example 1 purports to disclose an all-purpose preparation procedure which should generally apply to synthesizing anyone of the countless α-lipoic acid salts, regardless of the specific base chosen. The procedure comprises suspending one equivalent of the base in ethanol, heating the suspension at about 50°C to dissolve the base, and adding under stirring one equivalent of α-lipoic acid. Following cooling of the resulting solution, the salt would precipitate in crystalline form.

It can be easily shown that the aforesaid procedure is utterly inapplicable to the preparation of creatine lipoate: about none litres of alcohol are needed to dissolve on gram of creatine monohydrate (see The Merck Index, thirteenth edition, 2001, page 450) and its dissolution rate is only negligibly increased by heating.

Moreover, no solid crystalline product is obtained, but an oily sticky mass which strongly adheres to the reaction vessel walls.

In conclusion, EP 0 702 953 B1 does not disclose creatine lipoate nor a feasible process for producing it.

In the last year, the use of dietary supplements, nutraceuticals and health foods containing substances of natural origin as active ingredients has become more and more widespread, arousing the interest of ever wider consumers classes.

Creatine is but one of the natural products which, thanks to its physiologic activity, has brought about a major interest both in the scientific community and the consumers.

Creatine is an amino acid present in considerable amounts in the skeletal muscle tissue of vertebrates wherein about 2/3 thereof occurs as creatine phosphate.

Creatine is biosynthesized mainly in the liver and kidneys from three amino acids: glycine which provides the carbon skeleton, arginine which releases the amidino group and methionine which releases the methyl group. Creatine is excreted with urine as creatinine. Creatine can be taken with the diet since it is principally present in meat. However, in order to take 10 grams/day of creatine, 2.5 kg of meat should be eaten. The exogenous supply and endogenous biosynthesis must compensate for the daily turn-over of creatine to creatinine which in a 70-kg male subject can be estimated at about two grams.

The physiologic role of creatine is extremely important: principally in the skeletal muscle, but in the brain, liver and kidneys as well, creatine - by reversibly taking up ATP's phosphate groups - plays the role of reservoir of the energy-rich phosphate radicals. This reaction is critically important since ATP can not be stored in tissues in excess of a very limited threshold. It is creatine phosphate whose content in tissues is five times as much that of ATP, which provides for phosphate groups supply. Following a moderately wearying physical exertion, the creatine phosphate present in the skeletal muscle decreases in a far relevant amount than ATP does, thus showing that creatine phosphate rephosphorilates ADP as ATP becomes dephosphorilated.

When the rate of ATP's metabolic production exceeds ATP's utilization, this results in creatine phosphate formation. Creatine phosphate is, therefore, a reservoir of immediately available energy, suitable for counterbalancing energy demands exceeding ATP's synthesis rate in metabolic phosphorylation processes.

Creatine is mainly taken by athletes and sportsman insofar as it increases the skeletal musculature if its intake is accompanied by

lasting physical exertion. Creatine intake results in a lowering of fat while it enhances skeletal muscle. Recent researches have shown that the combined intake of creatine and carbohydrates enhances creatine effects owing to insuline production that is stimulated by simple sugars which likely play a role in creatine exportation to muscle cells.

Alpha-lipoic acid (thioctic acid; 1,2-Dithiolane-3-pentanoic acid) can occur both in oxidized and reduced form.

The adjacent sulphur atoms are decisive in imparting some unique and very important biochemical properties to the compound.

Studies carried out on the biochemical function of lipoic acid have shown that it is a catalyst endowed with high biological activity. It is a potent antioxidant, a chelating agent with scavenging activity on toxic substances, such as cadmium, mercury, lead and arsenic, a potent antidote to mushroom poisoning and a hypoglycemic agent.

It acts as catalytic coenzyme in energy-releasing metabolic processes.

In the last years, the antioxidant properties of lipoic acid, both in oxidized and reduced form (i.e. dihydrolipoic acid, DHLA) have aroused great interest. Lipoic and dihydrolipoic acid are in fact capable of scavenging hydroxyl radicals, singlet oxygen, hypochlorous acid and peroxy radicals.

At the Molecular Biology Department of Berkley University, one of the most outstanding world-wide research institute on antioxidant agents, lipoic acid has been categorized as the ideal antioxidant insofar as it possesses the following biochemical properties:

- it is both readily absorbable and bioavailable;

- it occurs in several organic structures: tissues, cells, extracellular fluids, various membranes, etc.;

- it interacts synergically with other antioxidants;

- it favourably affects gene expression.

Lipoic acid is the only substance capable of fulfilling all of the above-mentioned prerequisites, because of its ability to act as an antioxidant in fat- and water-soluble tissues. (Parker L. Witt E. Tritschler H. Alpha -lipoic acid as a biological antioxidant. Free Radic. Biol. Med. 1955; 19: 227-250).

This property is very important since it allows lipoic acid to act in all of organism structures ranging from blood and extracellular fluids prevailingly consisting of water to cellular and nuclear membranes prevailingly consisting of fats sensitive to lipid peroxidase, acting both inside and outside of cells.

Lipoic acid has a very low redox potential. Consequently, through its reduced form (dihydrolipoic acid, DHLA) it acts as a potent donor of electrons, thus scavenging free radicals and regenerating other antioxidants such as Vitamin E, Vitamin C and glutathione (Han D. Tritschler H, Packer L. Alpha-lipoic acid increases intracellular glutathione in human T-Lymphocyte Jurkat cell-line. Biochem Biophys Res Common. 1995; 207:258-264).

As regards its catalytic activity in energy-releasing processes, lipoic acid acts as co-enzyme in carbohydrates and fatty acids oxidation. This, reaction leads to ATP formation, a key compound in energy metabolism insofar as it "chemically" accumulates energy in the organism.

Recent studies have confirmed the aforesaid activities and have shown that an increase in glucose availability triggers an increase in energy production, an enhanced metabolism and ATP production in muscular tissues.

Regeneration from physical strains is also improved with allows better muscular performances to be achieved.

The intake of lipoic acid brings about, therefore, better muscular performances and a sharp decrease in fatty accumulations.

Creatine lipoate which synergistically combines the favourable pharmacological and physiological effects of creatine and lipoic acid mimicks insuline action enhancing glucose uptake. A fast-acting and practical agent is then made available for enhancing creatine absorption actually utilized by muscle cells [Halbertam M. et al., Diabetes 45(5): 659-666, 1996] and counteracting the free radicals action, achieving detoxication without provoking an increase in body weight, and improving cardiac and muscular performances.

In therapy creatine lipoate plays an important, useful role in the treatment of chronic fatigue syndrome.

A further object of the present invention is to provide a process for preparing creatine lipoate, which can be carried out on an industrial scale.

The process of the invention for preparing creatine lipoate comprises:

(a) preparing a mixture of equimolar amounts of α-lipoic acid and creatine monohydrate:

(b) heating the mixture till complete melting thereof, thus obtaining a clear molten phase;

(c) adding cyclohexane under stirring to the molten phase, thus obtaining a liquid mixture;

(d) cooling the liquid mixture to room temperature while keeping the mixture under stirring, thus obtaining a precipitate;

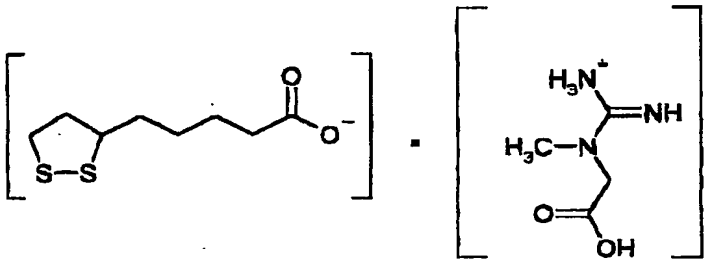
(e) filtering off the precipitate and drying it under vacuum.

In step (b) the mixture is preferably heated to about 75-80°C.

The following Example shows the preparation and physico-chemical properties of the novel salt according to the invention.

Example

Creatine lipoate (LC 101)



M.W. 337.45 $C_{12}H_{23}N_{34}S_2$

20.6 g (0.1 moles) of lipoic acid and 14.9 (0.1 moles) of creatine monohydrate were fed into a 250-ml two-necked round bottom flask equipped with a mechanical stirrer.

The reaction mixture was heated to 75°C and kept at this temperature for about 10 minutes till the reaction a mixture was completely melted.

Heating was then discontinued and to the thick, clear, yellowish liquid thus obtained 100 ml of cyclohexane were added, keeping the resulting mixture under stirring until its temperature reached room temperature. A solid precipitate was obtained which was filtered off and dried under vacuum overnight. 35 g (yield 98%) of a yellowish crystalline product were obtained.

Physico-chemical properties of the compound:

K.F. 0.8%;
M.P. 68-69°C
NMR: H = CD_3OD = 3.9 (2H,s,$\underline{CH_2}$-N); 3.8-3.4 (1H,q,CH-CH_2); 3.4-3 (2H,t, $\underline{CH_2}$-S); 2.9-2.6 (2H,q); 2.5-2.1 (2H,q, CH_2-CH_2); 2-1.8 (2H,t, $\underline{CH_2}$-COOH); 1.8-1.3 (4H,m, CH_2-$\underline{(CH_2)_2}$-CH_2-COOH)

Microanalysis	C%	H%	N%	S%
calculated	42.7	6.87	12.45	19
found	42.62	6.91	12.41	18.7

HPLC
Column Spherisorb Sax 5 μm (250 x 4.6 mm); t = 30°C;
Solvent H_2O + NaH_2PO_4-0.05M/CH_3CN (35:65);
Flow 1.0 ml/min Rt = Lipoic acid 4.06 minutes
Rt Creatine 7.68 min
Rate: Lipoic acid/Creatine = 60/40%

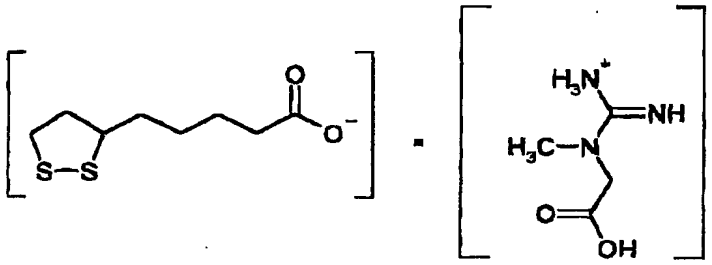
The compositions of the invention comprising creatine lipoate as active ingredient may also comprise, in addition to the usual pharmacologically acceptable excipients whose selection is within the reach of the average skilled expert in pharmacy, further active principles, aminoacids, antioxidants, mineral substances, vitamins and coenzymes.

Preferred, although non-limiting, examples of these further ingredients are taurine L-carnitine, acetyl L-carnitine, propionyl L-carnitine, coenzyme Q_{10} and the bioavailable forms of mineral substances such as selenium, magnesium and zinc.

The compositions can be administered in the form of tablets, chewable tablets, capsules, sachets, granulates, powders, syrups and drops. The compositions in unit dosage form comprise from about 100 to 1,000 mg, preferably from about 150 to 250 mg, of creatine lipoate.

Claims

1. Creatine lipoate of formula



2. A composition comprising creatine lipoate as active ingredient and a pharmacologically acceptable excipient.

3. The composition of claim 2, comprising at least one further ingredient selected from active principles, aminoacids, antioxidants, mineral substances, vitamins and coenzymes.

4. The composition of claims 3, wherein the further ingredient is selected from the group comprising taurine, L-carnitine, acetyl L-carnitine, propionyl L-carnitine, coenzyme Q_{10} and bioavailable compounds of selenium, magnesium and zinc.

5. The composition of claims 2-4 in the form of tablets, chewable tablets, capsules, sachets, granulates, powders, syrups and drops.

6. The composition of claims 2-5 in unit dosage form, comprising about 100-1.000 mg, preferably 150-250 mg, of creatine lipoate.

7. The composition of claims 2-6 for human consumption as dietary supplement, nutraceutical, health food, cosmetic or drug.

8. A process for preparing creatine lipoate which comprises:

(a) preparing a mixture of equimolar amounts of α-lipoic acid and creatine monohydrate:

(b) heating the mixture till complete melting thereof, thus obtaining a clear molten phase;

(c) adding cyclohexane under stirring to the molten phase, thus obtaining a liquid mixture;

(d) cooling the liquid mixture to room temperature while keeping the mixture under stirring, thus obtaining a precipitate;

(e) filtering off the precipitate and drying it under vacuum.

9. The process of claim 8 wherein in step (b) the mixture is heated to about 75-80°C.

INTERNATIONAL SEARCH REPORT

Intl ›nal Application No
PCT/IT 03/00316

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D339/04 C07C279/14 A61K7/48 A61K31/385 A61P39/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 702 953 A (ASTA) 27 March 1996 (1996-03-27) cited in the application claims 1-8	1-3
Y	WO 02 26206 A (BEIERSDORF) 4 April 2002 (2002-04-04) claims 1,4	1-3
Y	WO 02 02075 A (BEIERSDORF) 10 January 2002 (2002-01-10) claims 1,5	1-3
Y	FR 4 630 M (C.E.R.E.T.) 28 November 1966 (1966-11-28) page 1 -page 3	1-3

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2003	23/09/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Francois, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/IT 03/00316

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 702953	A	27-03-1996	DE	4433764 A1	28-03-1996
			AT	189387 T	15-02-2000
			AT	229333 T	15-12-2002
			CA	2158630 A1	23-03-1996
			DE	59507727 D1	09-03-2000
			DE	59510508 D1	23-01-2003
			DK	702953 T3	26-06-2000
			DK	947194 T3	24-03-2003
			EP	0702953 A2	27-03-1996
			EP	0947194 A1	06-10-1999
			ES	2144077 T3	01-06-2000
			ES	2189315 T3	01-07-2003
			GR	3033191 T3	31-08-2000
			HU	221843 B1	28-02-2003
			HU	75248 A2	28-05-1997
			JP	8104629 A	23-04-1996
			PT	702953 T	31-07-2000
			US	6348490 B1	19-02-2002
			US	5990152 A	23-11-1999
WO 0226206	A	04-04-2002	DE	10048260 A1	11-04-2002
			WO	0226206 A1	04-04-2002
			EP	1324745 A1	09-07-2003
WO 0202075	A	10-01-2002	DE	10032964 A1	24-01-2002
			WO	0202075 A1	10-01-2002
			EP	1296642 A1	02-04-2003
FR 4630	M		NONE		